placed over the existing labels. The laboratory-generated bar-coded number was unrelated to the original label information provided by the Sponsor. The laboratory personnel who applied the new labels were not further involved in the study. The samples were randomized using a random number table (Appendix 2). Samples were maintained at –20 C until the afternoon prior to testing. On that afternoon, the samples scheduled for testing the next day were placed at 4 C overnight. The following morning, these samples were brought to room temperature.

Laboratory personnel were provided with a copy of the product package insert for each test kit. Laboratory personnel were trained by the Sponsor in the use of each test kit according to the exact instructions in the product package insert. Training occurred on July 18, 2011. Samples were tested between July 18 and July 26, 2011. Testing occurred without involvement of the Sponsor, and no Sponsor personnel were present during testing. Testing was not performed on July 21, 2011 or July 25, 2011 due to lack of availability of the full complement of laboratory personnel. Each sample was tested using the same procedure:

1. The laboratory coordinator inverted the sample tube 5 or 6 times.

2. Each different pregnancy test kit was used to test the sample using the method set forward in the product package insert. The order in which the test kits were selected was varied from one urine sample to the next. If a test kit failed to activate or failed to produce a reading after dipping, the kit was replaced by another of the same test kit type.

3. After dipping the test kit into the sample for the specified time period, the laboratory coordinator waited for the specified length of time before the test was read.

4. The test kit and the sample vial were presented to each of three readers in varied order. The readers did not observe one another reading the test kit and did not communicate with one another.

5. Each reader was seated at a computer work station equipped with a barcode reader. The reader scanned the sample label for identification of the sample following which he or she read the test kit as positive, negative, or quantity not sufficient (QNS; explained below). Positive corresponds to pregnant and negative corresponds to not pregnant, and readings were determined according to the product package labeling. Each reader had a card to which were affixed barcoded labels corresponding to each of the three possible results, and results were entered by scanning the appropriate bar code with the bar code reader.

6. When a sample vial contained too low a volume for testing all test kits using the dip method, a 1-mL aliquot was pipetted using a calibrated pipette onto the sample wick of each device in the order that had been assigned to the devices. If there was inadequate urine to deliver 1-mL to a test kit, that kit was delivered to the reader in its untested condition, that is, bearing no result. Readers were instructed to read those kits as QNS.

7. Each reader sat at the same work station for each test. The same laboratory coordinator and readers were involved throughout the entire study. Laboratory personnel, including the laboratory coordinator, were unaware of the study objective, the anticipated number of positive

pregnancy tests, the number, sex, or pregnancy status of the subjects who provided urine samples, and the identity of the study Sponsor.

8. After the completion of all tests, test results were provided by the laboratory (Appendix 3) and tabulated by the principal investigator for analysis according to the data from the original sample label, which permitted identification of the cycle day relative to EMP for each subject (Appendix 4). Tests kits were considered positive or negative based on agreement of at least two of the three readers.

9. Data analysis was conducted in Excel for each pregnancy test device according to the percent positive tests for each day relative to EMP. A kappa statistic was calculated using the Fleiss method to represent the agreement of the three readers across all samples for each test device.

Protocol amendments and deviations

There were no protocol amendments. The pipette method for low-volume samples was not described in the original protocol but was agreed by the Sponsor and principal investigator prior to the beginning of the study.

Study deviations

There were no deviations.

Results

Testing of each sample according to the product package insert was possible using each test kit with the exception of the following:

- One sample from EMP–5. This sample was tested using the pipette method for the First Response® Gold Digital Pregnancy Test (old) and the First Response® Early Result Pregnancy Test, because these two tests were the first two in the order for that sample. The other test kits (the First Response® Gold Digital Pregnancy Test (new) and the Clearblue® Easy test) were not tested for this sample from EMP –5.
- The pipette method was used for an additional three samples tested on all four test kits: two from EMP–2 and one from EMP.

No invalid test results were displayed. There were some devices that failed to activate; these devices were replaced as per protocol. There was a high level of agreement among the readers with kappa statistics as follows. Calculations appear in Appendix 5.

Test kit	Kappa
First Response® Gold Digital Pregnancy Tests (the old test kit)	0.997
First Response® Gold Digital Pregnancy Tests (the new test kit)	0.997
Clearblue® Easy Digital Pregnancy Test	0.995
First Response® Early Result Pregnancy Test	0.948

Tables 1–4 present the percentage of positive test kits on each cycle day relative to EMP. Figure 1 shows a side-by-side comparison of the four test kits. The day on which more than 50% of test kits became positive was EMP–6 for the First Response® Early Result Pregnancy Test and EMP–5 for the other test kits. Exclusion of the samples that were tested using the pipetting method had no appreciable effect on the results.

Table 1. Detection of hCG in Conceptive Cycles Relative to Expected Menstrual Period (EMP) and LH Peak Using Clearblue® Easy Digital Pregnancy Test

Days from EMP	–14	–13	–12	–11	–10	–9	–8	–7	–6	–5	–4	–3	–2	–1	EMP	+1
Days from LH	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10	+11	+12	+13	+14	+15	+16
No. of samples	1	1	4	7	18	26	40	47	48	47[a]	48	48	48	47	48	45
Number positive	0	0	0	0	0	0	2	9	17	27	36	42	47	46	47	44
Percent positive	0	0	0	0	0	0	5.0	19.1	35.4	57.4	75.0	87.5	97.9[b]	97.9	97.9[b]	97.8
% Positive excluding pipetted samples	0	0	0	0	0	0	5.0	19.1	35.4	57.4	75.0	87.5	97.8	97.9	97.8	97.8

[a]One of 48 samples had insufficient quantity for testing
[b]Two samples on EMP–2 and one sample on EMP were tested using the pipetting method. All three pipetted samples were positive.

Table 2. Detection of hCG in Conceptive Cycles Relative to Expected Menstrual Period (EMP) and LH Peak Using First Response® Early Result Pregnancy Test

Days from EMP	–14	–13	–12	–11	–10	–9	–8	–7	–6	–5	–4	–3	–2	–1	EMP	+1
Days from LH	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10	+11	+12	+13	+14	+15	+16
No. of samples	1	1	4	7	18	26	40	47	48	48	48	48	48	47	48	45
Number positive	0	0	0	0	0	3	9	18	30	44	46	46	48	47	48	45
Percent positive	0	0	0	0	0	11.5	22.5	38.3	62.5	91.7[a]	95.8	95.8	100[a]	100	100[a]	100
% Positive excluding pipetted samples	0	0	0	0	0	11.5	22.5	38.3	62.5	91.5	95.8	95.8	100	100	100	100

[a]Two samples on EMP–2 and one sample each on EMP–5 and EMP were tested using the pipetting method. All four pipetted samples were positive.

Table 3. Detection of hCG in Conceptive Cycles Relative to Expected Menstrual Period (EMP) and LH Peak Using First Response® Gold Digital Pregnancy Tests (New Test Kit)

Days from EMP	–14	–13	–12	–11	–10	–9	–8	–7	–6	–5	–4	–3	–2	–1	EMP	+1
Days from LH	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10	+11	+12	+13	+14	+15	+16
No. of samples	1	1	4	7	18	26	40	47	48	47[a]	48	48	48	47	48	45
Number positive	0	0	0	0	0	1	4	11	23	29	40	45	47	47	48	45
Percent positive	0	0	0	0	0	3.8	10.0	23.4	47.9	61.7	83.3	93.8	97.9[b]	100	100[b]	100
% Positive excluding pipetted samples	0	0	0	0	0	3.8	10.0	23.4	47.9	61.7	83.3	93.8	97.8	100	100	100

[a]One of 48 samples had insufficient quantity for testing
[b]Two samples on EMP–2 and one sample on EMP were tested using the pipetting method. All three pipetted samples were positive.

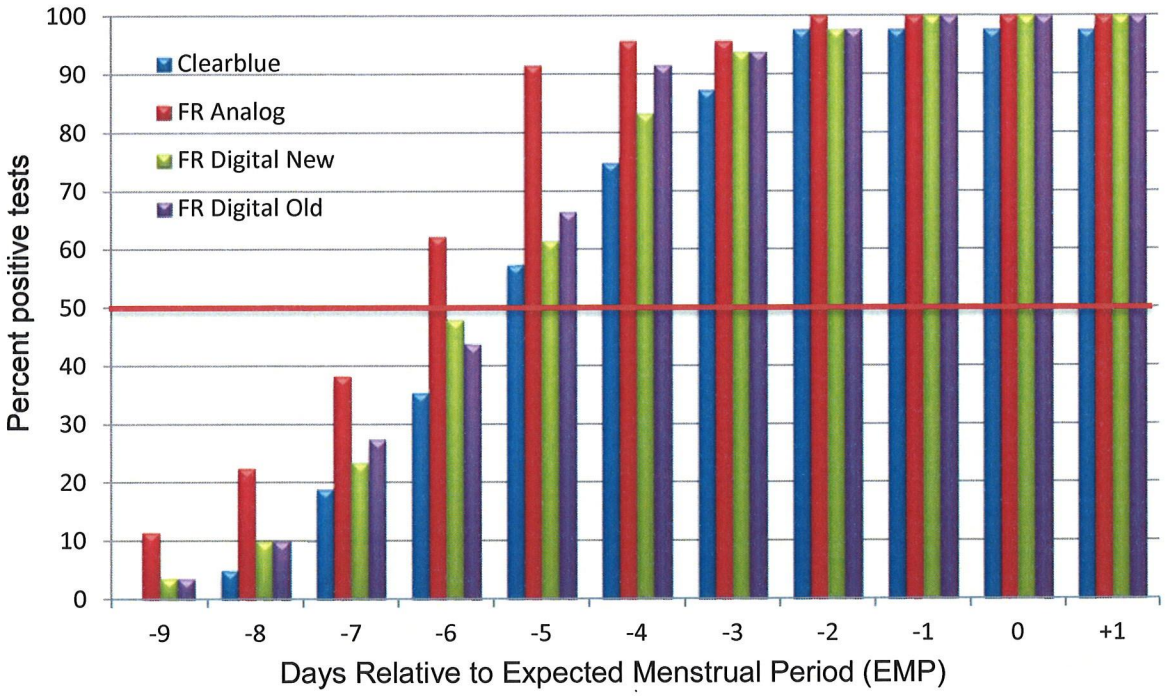
Table 4. Detection of hCG in Conceptive Cycles Relative to Expected Menstrual Period (EMP) and LH Peak Using First Response® Gold Digital Pregnancy Tests (Old Test Kit)

Days from EMP	–14	–13	–12	–11	–10	–9	–8	–7	–6	–5	–4	–3	–2	–1	EMP	+1
Days from LH	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10	+11	+12	+13	+14	+15	+16
No. of samples	1	1	4	7	18	26	40	47	48	48	48	48	48	47	48	45
Number positive	0	0	0	0	0	1	4	13	21	32	44	45	47	47	48	45
Percent positive	0	0	0	0	0	3.8	10.0	27.7	43.8	66.7[a]	91.7	93.8	97.9[a]	100	100[a]	100
% Positive excluding pipetted samples	0	0	0	0	0	3.8	10.0	27.7	43.8	68.1	91.7	93.8	97.8	100	100	100

[a]Two samples on EMP–2 and one sample each on EMP–5 and EMP were tested using the pipetting method. The pipetted sample on EMP–5 was negative and the remainder of the pipetted samples were positive.

Figure 1. Comparison of the four pregnancy tests

"Clearblue" is Clearblue® Easy Digital Pregnancy Test, "FR Analog" is the First Response® Early Result Pregnancy Test, "FR Digital" is First Response® Gold Digital Pregnancy Tests



Conclusions

Using urine samples from 48 conceptive cycles in women, the First Response® Early Result Pregnancy Test, the old and new First Response® Gold Digital Pregnancy Tests, and the Clearblue® Easy Digital Pregnancy Test were read by a panel of three masked readers. More than 50% of First Response® Early Result Pregnancy Test results were positive 6 days before the expected menstrual period. More than 50% of the other pregnancy tests (the old and new First Response® Gold Digital Pregnancy Tests and the Clearblue® Easy Digital Pregnancy Test) became positive one day later, 5 days before the expected menstrual period.